Oey, Jan

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Glucuronide validation data (44/20)

Subject:







chrom.doc

line2.doc

Additional glucuronide data as requested:

Representative chromatogram of deconjugated nicotine and cotinine

glucuronides (chrom.doc).

- 2) Calibration curves for nicotine and cotinine glucuronides (line1.doc and line2.doc).
- Freeze/thaw data (3 additional cycles) and room temperature stability (24 hours) was supplied with the original validation data on 19 Oct.

Extract stability of glucuronides was not performed for this study.

because the extracted glucuronides are deconjugated during the extraction procedure. Therefore extract stability is covered by

data generated during the aglycone validation study (44/6).

4) Analytical method summary:

Urine samples are thawed at nom. 25xC and spiked with internal standards. The samples are then deconjugated using B-glucuronidase

at nom. 37xC for 18-22 hours. The aglycones are extracted from the urine

using HCX SPE cartridges and the extracts submitted for analysis

by

LC-MS/MS.

Best regards,

Jill

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